Lamellate Structures in the Nucleolus of the Cellular Slime mold *Acrasis rosea*[1]

Hans R. Hohl and Susan T. Hamamoto[2]

During a study on the morphogenesis of *Acrasis rosea,* a cellular slime mold, we have encountered lamellar structures appearing as part of the nucleolus of spores and stalk cells in the fruiting body. This report describes in detail these structures, their occurrence, and their possible nature.

Materials and Methods

Acrasis rosea originally obtained from Dr. A. Kahn, Syracuse University, was grown on Difco cornmeal-dextrose agar supplemented with 0.1% yeast extract (Weitzman, 1962) with *Rhodotorula mucilaginosa* as food organism. Myxamoebae and fruiting bodies were most successfully prepared for electron microscopy by one of the following two methods: (1) Fixation for 1 hour in osmium vapor, followed by 1 hour in 2.5% phosphate-buffered glutaraldehyde at pH 7.3, and post-fixation for 1 hour in 1% phosphate-buffered osmium tetroxide at pH 7.3. (2) Fixation for ½ hour in Karnovsky's (1965) cacodylate-buffered glutaraldehyde-paraformaldehyde at pH 7.0, diluted to ¼ (personal communication from Dr. S. Ito, Harvard University), followed by fixation in 1% Palade's osmium tetroxide at pH 7.3 for ½ hour. For both methods dehydration was carried out in ethyl alcohol followed by propylene oxide, and the specimen was then embedded in epon. In some cases the tissue was incubated in 0.5% uranyl acetate in 70% ethyl alcohol for ½ hour during dehydration. Sections were cut on an LKB ultramicrotome, post-stained in lead citrate and viewed in a Hitachi HU-11A electron microscope.

For demonstration of RNA, glutaraldehyde-paraformaldehyde fixed (½ hour, 4°C, pH 6.0) samples were treated with 0.7 mg/ml RNAase (heated for 10 minutes at 90°C prior to use). After incubation, the cells were washed with 5% trichloracetic acid for 30 minutes at 0°C, postfixed in osmium tetroxide, and embedded as described above.

For histochemical purposes at the light-microscope level the cells were fixed for 10 minutes in acetone at 4°C. The cells were stained for (1) RNA using pyronin Y according to Kurnick (1955), for (2) DNA and RNA with acridine orange followed by fluorescence microscopy, and for (3) protein with mercury bromphenol blue following the procedure outlined by Pearse (1960). Controls were treated with DNAase or RNAase (Loh and Soergel, 1965) before staining.

Results

The nucleolus of the myxamoebae of *Acrasis rosea* appears to be built from three morphologically distinct components. The most striking, and the one of particular interest for our discussion, consists of a series of roundish masses of granular material (Figs. 1 and 2). These masses are either dispersed as separate units within the nucleus (Fig. 2) or condensed into large aggregates (Fig. 3). Each mass is made up of a large number of electron dense granules which stain heavily with lead citrate and are comparable in size to cytoplasmic ribosomes. The granules are more densely packed at the periphery of each clump, the central part of which then appears as a light core revealing a somewhat fibrous matrix.

The second component has not been observed frequently. It appears as an intensely stained, homogeneous body containing electron transparent cavities and is always situated within the first component (Figs. 1 and 5). The third component of the nucleolus consists of a large

[1] This research was supported by a research grant (GM 11758-03) from the National Institutes of Health of the U. S. Public Health Service.

[2] Pacific Biomedical Research Center and Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822. Manuscript received May 23, 1967.

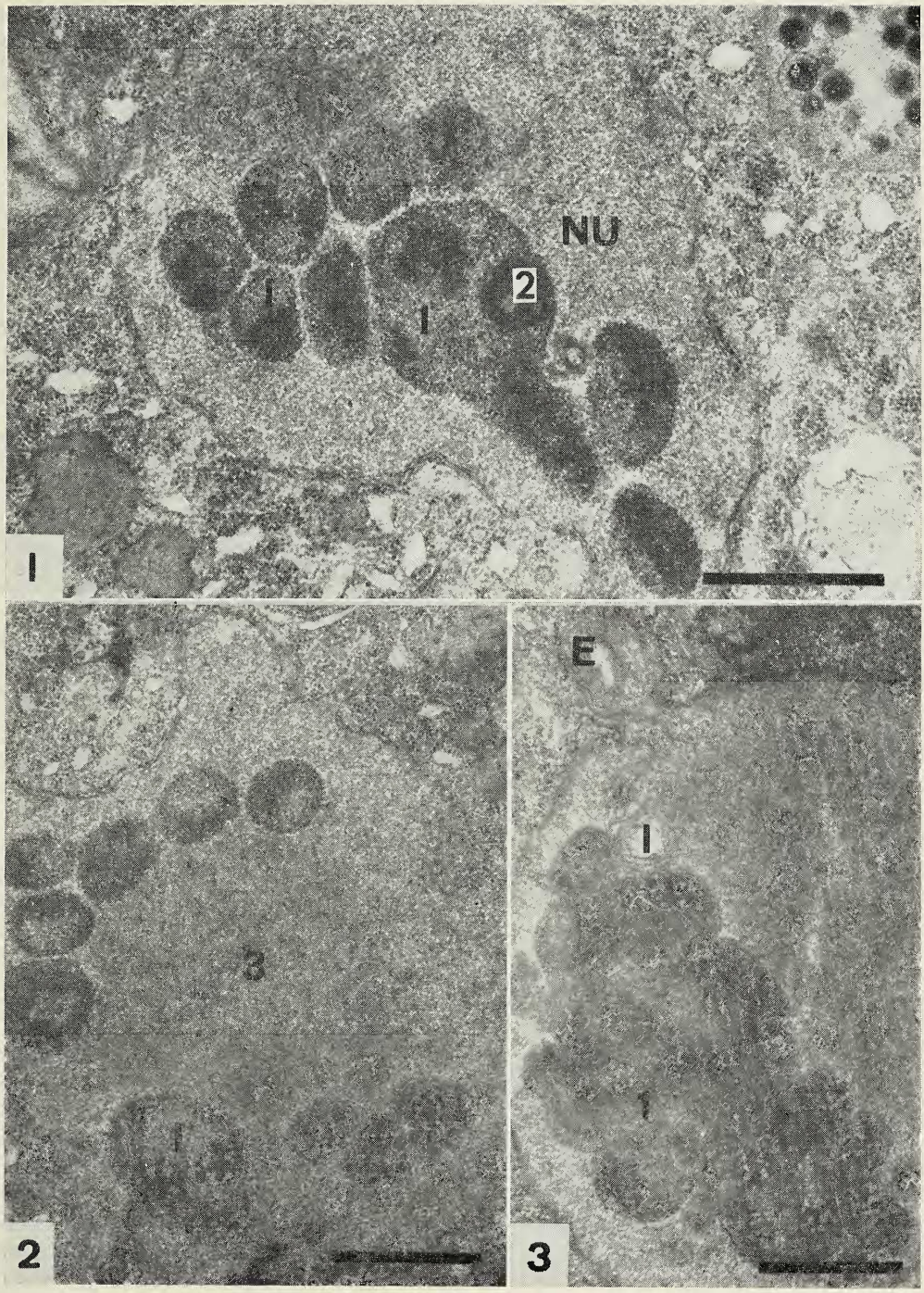

Fig. 1. Part of a cell of *Acrasis rosea* showing nucleus (NU) with two components of the nucleolus (1,2) as described in the text. × 25,000. The black bar in all pictures represents 1μ.

Fig. 2. Nucleus of *Acrasis rosea* with components 1 and 3 of nucleolus. Note lighter and darker areas within component 1. × 20,800.

Fig. 3. Example of component 1 aggregated into a large mass. Cytoplasmic invagination (*I*) and nuclear evagination (*E*) are seen at upper left. × 20,000.

roundish mass of finely granular material somewhat more dense than the rest of the nucleus but considerably less dense than the first component (Fig. 2).

In the resting stages, that is, in the stalk cells and the spores, the first granular component often contains lamellar elements which are stacked in parallel fashion (Fig. 4). The number of lamellae per stack has been observed to vary from 1 to 12. The lamellae can be flat or curved, may cross through the middle of the granular component (Figs. 4 and 5), or may follow its contour (Figs. 6 and 7). Yet they never extend beyond the granular mass. The lamellae are separated by a constant distance of approximately 150 Å. The constant thickness of the lamellae together with the constant spacing conveys to the whole structure a regularity of appearance (Fig. 4).

Each lamella consists of a single layer of granules (Figs. 1, 6, and 7) apparently identical to the granules making up the first component (Figs. 1, 2, and 8). In fact, the layers are often continuous with the granular component (Figs. 8 and 9) and are always closely adjacent to it. Oblique sections through the stacks provide additional evidence that the layers are built from granules and not, for example, from a filamentous component (Fig. 8). Furthermore, we see that the granules are not oriented in any particular way but are packed rather tightly, though randomly, within the planes.

By applying the histochemical tests described, ribonucleoprotein-containing areas can be demonstrated within the nucleus comparable in size and location with the components described at the electron microscope level. This suggests that the granular masses contain ribonucleoprotein and are probably aggregates of ribosomes or ribosomal precursors. The nucleolus has been shown to be a source of ribosomes (Birnstiel, Chipchase, and Hyde, 1963). Our best evidence supporting this idea is obtained from the electron microscope study of RNAase treated cells in which the granular component has largely faded (Fig. 10).

DISCUSSION

The lamellae described in this report bear a certain resemblance to the "core" structures of spermatocytes (Moses, 1956) as well as to the chromatoid bodies in parasitic protozoa and in germ cells of plants and animals (Barker, 1963). In the case of the "cores" a close similarity can be noted between the outer layer of the complex cores described by Schin (1965) and our lamellae. In both cases the layers are granular, show continuity with the nucleolus, and are often attached to or partly surrounded by the latter (Schin, 1965; Guénin, 1965). In the case of *Acrasis,* however, all the other components of the "core" structure are lacking; also, the cells are at a resting stage and no division of cells or nuclei occurs.

In the chromatoid bodies of *Entamoeba invadens,* granules identified as ribonucleoprotein particles also are arranged in layers. A closer examination of those lamellae reveals, however, that the granules within the layers form helices (Siddiqui and Rudzinska, 1963) and thus differ from the arrangement described here for *Acrasis.*

All of these examples demonstrate the capacity of ribonucleoprotein granules to assemble into orderly structures. In our case particularly, we attribute this to a process of spontaneous self assembly within or at the periphery of a highly concentrated mass of granules in a state of low metabolic activity.

The fact that the lamellae have so far been observed only in resting cells, and then not with regularity, indicates that they do not represent an organelle involved in an essential function.

SUMMARY

Lamellate structures have been demonstrated within the nucleoli of spores and stalk cells of the slime mold *Acrasis rosea.* The lamellae, numbering up to about 12, are separated from each other by about 150 Å and are arranged in a parallel fashion to form stacks which either cross the main nucleolar mass or line it peripherally. Each lamella is composed of a single layer of granules apparently identical to the ones making up the bulk of the nucleolus. The granules are fairly tightly but randomly packed within the layer. The lamellate structures are interpreted to represent an arrangement of ribosomal type particles formed from a concentrated pool of particles by a process of self assembly.

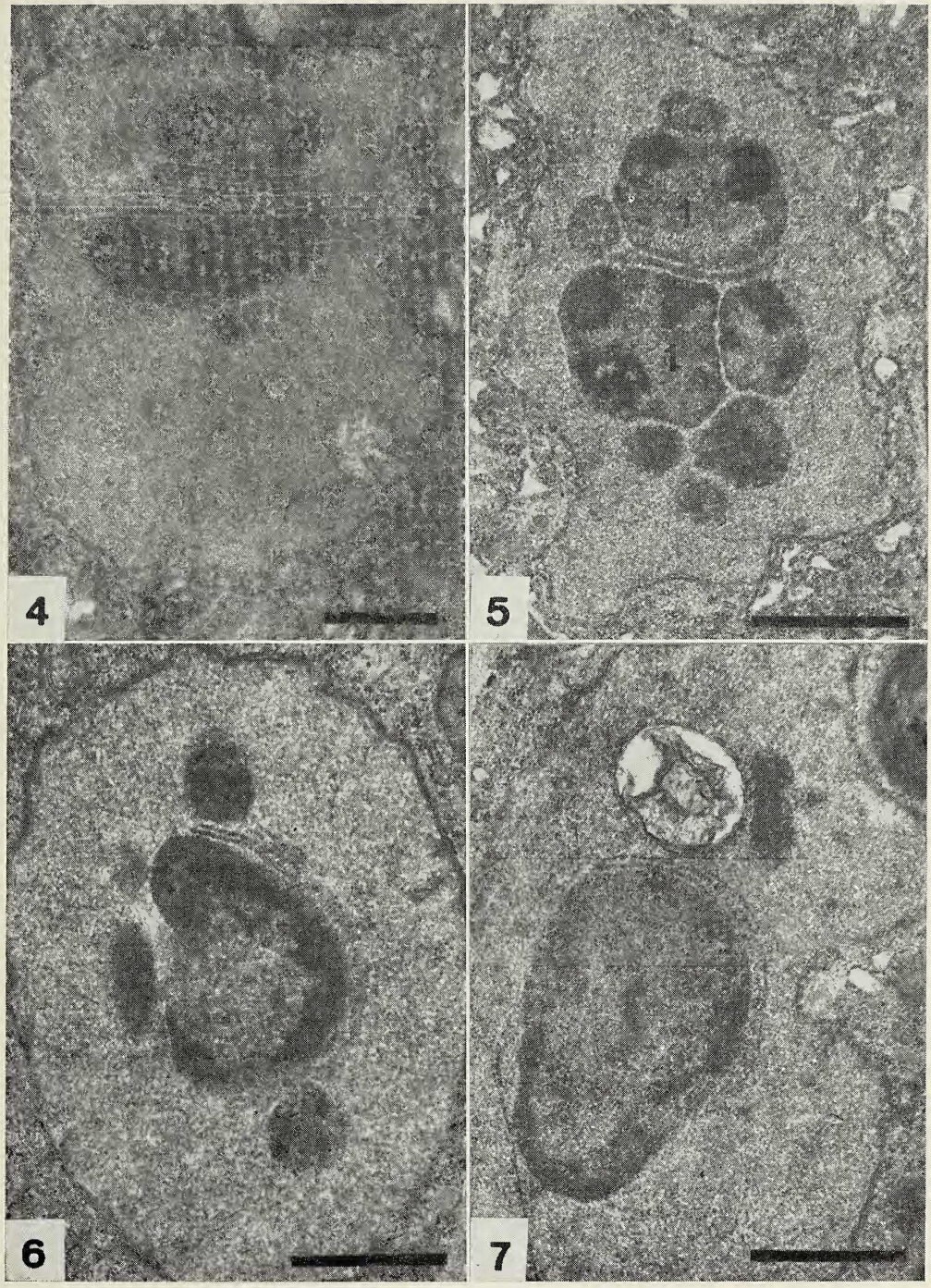

Fig. 4. Nucleolus of *Acrasis rosea* with a series of parallel lamellae transversing it. × 16,000. The black bar in all pictures represents 1μ.

Fig. 5. Massed component 1 of nucleolus with lamellar material in between portions of mass. × 22,000.

Fig. 6. Nucleolus with lamellae along contours. Lamellae are sectioned tangentially in parts. × 22,000.

Fig. 7. Nucleolus with peripheral lamellae. × 22,000.

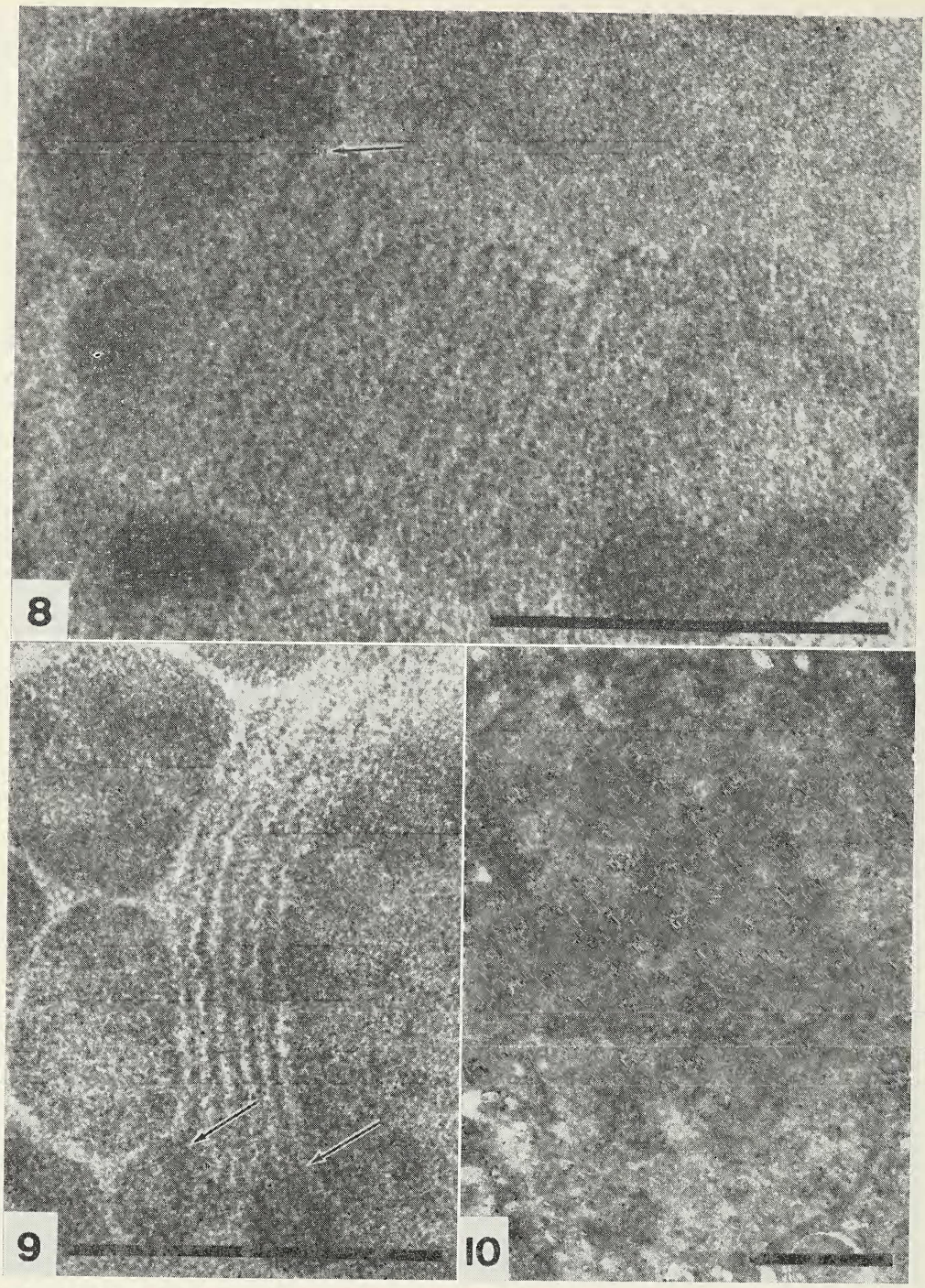

FIG. 8. Higher magnification of lamellar material cut obliquely. The lamellae appear to be made up entirely of granules and seem to be continuous with dark mass of component 1 (*arrow*). × 56,000. The black bar in all pictures represents 1μ.

FIG. 9. Continuity between lamellar material and component 1 of nucleolus (*arrows*). × 53,000.

FIG. 10. Nucleus after treatment with RNAase. The nucleolar masses have largely faded. × 20,000

We would like to thank Dr. Hans Ris for advice and Dr. Philip Loh for the use of the fluorescence microscope.

REFERENCES

Barker, D. C. 1963. A ribonucleoprotein inclusion body in *Entamoeba invadens*. Z. Zellforsch. 58:641–659.

Birnstiel, M. L., M. I. H. Chipchase, and B. B. Hyde. 1963. The nucleolus, a source of ribosomes. Biochim. Biophys. Acta 76: 454–462.

Guénin, H. A. 1965. Obsérvations sur la structure submicroscopique du complexe axial dans les chromosomes meiotiques chez *Gryllus campestris* L. et *G. bimaculatus* de Geer (Orthopt. Gryll.). J. Microscopie 4:749–758.

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27: 137A–138A.

Kurnick, N. B. 1955. Pyronin Y in the methyl-green-pyronin stain. Stain Technol. 30:213–230.

Loh, P. C., and M. Soergel. 1965. Growth characteristics of Reovirus type 2: actinomycin D and the synthesis of viral RNA. Proc. Natl. Acad. Sci. 54:857–863.

Moses, M. J. 1956. Chromosomal structure in crayfish spermatocytes. J. Biophys. Biochem. Cytol. 2:215–218.

Pearse, A. G. E. 1960. Histochemistry. 2nd ed. Little, Brown and Company, Boston. 998 pp.

Schin, K. S. 1965. Core-Strukturen in den meiotischen und post-meiotischen Kernen der Spermatogenese von *Gryllus domesticus*. Chromosoma 16:436–452.

Siddiqui, W. A., and M. A. Rudzinska. 1963. A helical structure in ribonucleoprotein bodies of *Entamoeba invadens*. Nature 200: 74–75.

Weitzman, I. 1962. Studies on the nutrition of *Acrasis rosea*. Mycologia 54:113–115.